vircell
MICROBIOLOGISTS

CELL LINES CATALOGUE

VIRCELL cell lines are available in ready-to-use presentations (Shell Vials and Tubes) and in other presentations for preparing tubes and plates (Flasks and Suspensions).

Shell Vials	Tubes	Suspensions	Flasks
No.: 24 pcs Dim.: 16 x 50 mm Vol.: 6 ml	No.: 24 pcs Dim.: 16 x 120 mm Vol.: 10 ml	Vol.: 30 ml (10^6 cells/ml)	75 or 150 cm^2

CELL LINES SOURCE

All our cell lines are grown and preserved in liquid nitrogen after the undergoing controls for Mycoplasma, fungal and bacterial contamination as mentioned below.

Cell cultures are renewed from the liquid nitrogen store every six months. Semi continuous cell lines are renewed before they reach suboptimal passages.

CELL QUALITY CONTROLS

Mycoplasma control

All cell batches and subcultures are tested for mycoplasma contamination by Hoechst staining and by PCR. Any lot or subculture in which mycoplasma is detected will be discarded.

Bacterial or fungal contamination control

Samples from every subculture or production lot are inoculated in bacterial broths and incubated at 2 temperature ranges (room temperature and 34-38°C). Controls will be monitored for two weeks prior to shipment. Any lot or subculture in which contamination is detected will be discarded.

Cell viability control

All cell batches and subcultures are microscopically screened during build up and production, for the absence of cytopathic effect and the presence of the adequate cell morphology. Besides these controls, every shell-vial, tube and flask is examined under the microscope on the day of shipment in order to detect any contamination and to ensure the correct morphology and confluence of the cell monolayer.

If any cell lot shipped to a customer does not fulfill the aforementioned requirements, the customer is notified and the cultures are replaced free of charge.

ASSAY PROCEDURE

Cell suspensions

- Upon receipt disperse the cells by passing them through a sterile pipette fitted with cotton at the upper end to avoid contamination.

- Mix 100 µl of the cell suspension with 20 µl of a 0,1% trypan blue solution. Count the cells in a Neubauer chamber excluding the blue-stained non-viable cells. The amount of growth medium that should be added to the suspension will be given by the following formula: [(No. of cells counted in a corner square x Suspension volume x 12,000)/ intended final cell concentration (usually 75-100 x 10^3 cells /ml for MRC-5)] - suspension volume.

  Example: We have got 30 ml of cell suspension and we count 115 cells in a square corner of the Neubauer chamber, of which 5 are stained blue. In order to adjust the final cell concentration to 100,000 cells/ml we should add: [(110 x 12,000 x 30)/ 100,000]- 30= 366 ml of growth medium.

- Shell vials are dispensed at 1 ml per vial and tubes at 2 ml per tube.

Adherent cells in flasks

- Remove the medium from the flask.
- Wash three times the monolayer with 5-10 ml sterile phosphate buffered saline (PBS) pH 7.5 preheated at 37ºC.
- Add 5-6 ml of sterile trypsin-EDTA mixture (0,12% trypsin and 1/4000 EDTA in PBS) preheated at 37ºC.
- After a few seconds, examine the cell monolayer against the light. When it turns opaque and the cells start detaching, dispose of the trypsin. Alternatively, the monolayer may be observed under the microscope until the cells start to separate.
- Tap the flask until the monolayer completely detaches.
- Add 10-15 ml of sterile growth medium preheated at 37ºC.
- Disperse cells by passing them through a sterile pipette fitted with cotton at the top end to avoid contamination.
- Add enough growth medium according to the number of flasks in which the cell suspension is going to be seeded. If a specific cell concentration is required, please refer to the previous section.

Shell vials and tubes

Examine the cells upon receipt, paying particular attention to the temperature, monolayer confluence, presence of contaminants, pH and monolayer quality. The medium contains phenol red, so a salmon red colour indicates pH between 7.2 and 8.9, which is the optimal range for culture maintenance; a yellow colour reveals an acid pH and may indicate cell contamination or overgrowth; a deep pink colour indicates an alkaline pH, probably due to the loss of CO_2 from the tube caused by a crack or a loose cap.

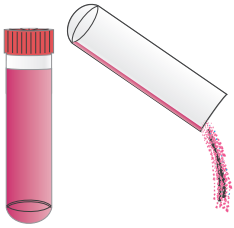
The maintenance medium of the tubes contains antibiotics to prevent fungal or bacterial contamination. If cells arrive in bad conditions or tubes are turbid, please notify us within the first 48 hours after delivery and we will replace them as soon as possible.

Place shell-vials in a standard laboratory rack and tubes in a 5º tilted rack with the monolayer covered by the medium and keep them at 34-38ºC until the moment of use. For best results in sensitivity and specificity, follow all the steps of the procedure described below and use appropriate staining reagents. VIRCELL, SL will not accept liability for the incorrect use of its cell lines.

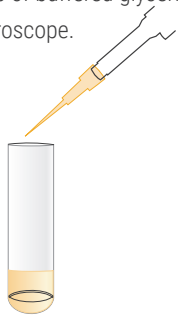
SUMMARY OF THE PROCEDURES

Traditional cell culture technique

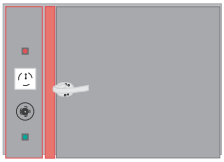
1. Incubate vials at 37ºC until time of use.
2. Remove the medium.
3. Add 100-200 µl of inoculum for each tube to be inoculated.
4. Incubate at 37°C for 1 hour.
5. Remove the inoculum and add 2 ml of MEM with 2% FBS. Incubate at 37ºC, examining periodically.
6. In the event of the appearance of CPE or after 7 days of incubation, break the monolayer with a Pasteur pipette and collect cells with the culture medium. Centrifuge and prepare slides from the pellet.
7. Fix with methanol or acetone and stain with fluorescein-linked antibody.
8. Wash with PBS. Dry and add a drop of buffered glycerin.
9. Observe under a fluorescence microscope.



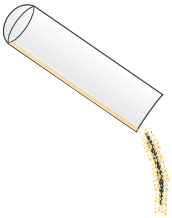
1. Remove the medium



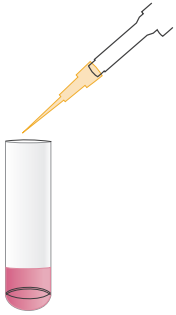
2. Inoculate 200 µl of sample



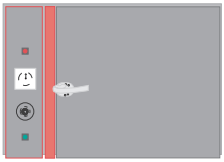
3. Incubate at 37º C for 1 hour



4. Remove the inoculum



5. Add 2 ml of growth medium



6. Incubate at 37º C

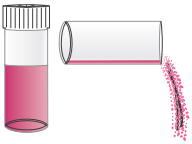
Shell vial technique

1. Incubate the vials at 34-38ºC until scheduled for use.
2. Remove the medium and inoculate 200 µl of the sample.
3. Centrifuge the tubes at 700 g for 45 minutes.
4. Keep at 34-38ºC for 1 hour.
5. Remove the inoculum and add 1 ml of MEM containing 2% of FBS.
6. Incubate for 24-48 h at 34-38ºC.
7. Dispose of the medium and fix for 10 minutes with methanol. If acetone is used, cool the acetone previously at temperature of -20ºC or lower and perform the fixation for 10 minutes at the same temperature.
8. Remove the cover slip by punching the bottom of the vial with a red-hot needle. Pick the cover slip with forceps (be careful for not to damage the cell monolayer) and air-dry it.
9. Adhere to a slide with DPX with the cell monolayer facing upwards (the cell-containing side appears opaque under light).Press slightly the cover slip against the slide with the help of a pipette tip to avoid bubbles.
10. Stain according to the instructions of the kit used for the detection step.

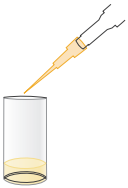
Chlamydia technique

1. Remove the culture medium.
2. Inoculate 200 µl of sample (urethral, cervical, conjunctive exudate...).
3. Centrifuge tubes at 1500 g for 60 minutes.
4. Keep at 37ºC for 1 h.
5. Remove the inoculums and add 1 ml of growth medium for Chlamydia.
6. Incubate for 48 hours at 37ºC.
7. Dispose of the medium and fix for 10 minutes with methanol.
8. Remove the cover slip.
9. Adhere to a slide with DPX.
10. Add 25 µl of FITC- labelled monoclonal antibody and incubate for 30 min at 37ºC in a humid chamber in the darkness.
11. Wash with PBS for 5 minutes.
12. Dry and add one drop of buffered glycerine. View under the fluorescence microscope.

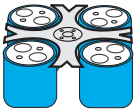
Shell vials inoculation



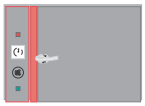
1. Remove the culture medium



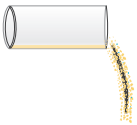
2. Inoculate 200 µl of the sample



3. Centrifuge at 700g for 45 min

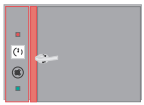


4. Keep 1 hour at 37ºC



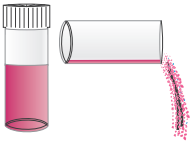
5. Remove the inoculum

6. Add 1 ml of growth medium

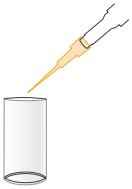


7. Incubate at 37ºC for 24 - 48 hours

Shell vial staining

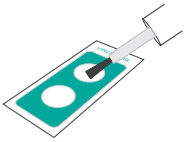


1. Remove the culture medium

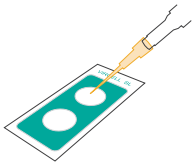


2. Fix for 10 min with methanol/acetone

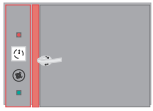
3. Remove the coverslip



4. Adhere the coverslip to a slide with DPX

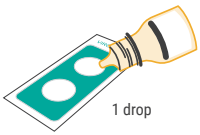


5. Add the FITC- monoclonal antibody



6. Incubate at 37ºC for 30 min

7. Wash with PBS for 10 minutes



8. Add glycerin

9. View with fluorescence microscope

CELL LINES CATALOGUE

Cell line	Shell Vials	Tubes	Flask	Suspensions
A-549	VSA5	VTA5	FTA5	SA5
B95-8			FTB95	
BGM	VSBG	VTBG	FTBG	
BHK 21			FTBH	
HEp-2	VSHE	VTHE	FTHE	
L-929 (mouse)	VSL9	VTL9	FTL9	
LLC-MK2	VSLL	VTLL	FTLL	
McCoy	VSMC		FTMC	
MDCK	VSMD	VTMD	FTMD	SMD
MDCK-SIAT1			FTMS	
MRC-5	VSMR	VTMR	FTMR	SMR
RD	VSRD	VTRD	FTRD	
Vero	VSVE	VTVE	FTVE	SVE
Vero E6	VSV6	VTV6	FTV6	

For custom cell lines or formats, please contact our Sales Department (info@vircell.com)

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